CaMKII inhibition in human primary and pluripotent stem cell-derived chondrocytes modulates effects of TGFbeta and BMP through SMAD signaling.

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Public Summary:
Abstract OBJECTIVE: Upregulation of calcium/calmodulin-dependent kinase II (CaMKII) is implicated in the pathogenesis of osteoarthritis (OA) and reactivation of articular cartilage hypertrophy. However, direct inhibition of CaMKII unexpectedly augmented symptoms of OA in animal models. The role of CaMKII in OA remains unclear and requires further investigation. METHODS: Analysis of CaMKII expression was performed in normal human and OA articular chondrocytes, and signaling mechanisms were assessed in articular, fetal and Pluripotent Stem Cell (PSC)-derived human chondrocytes using pharmacological (KN93), peptide (AC3-I) and small interfering RNA (siRNA) inhibitors of CaMKII. RESULTS: Expression levels of phospho-CaMKII (pCaMKII) were significantly and consistently increased in human OA specimens. BMP2/4 activated expression of pCaMKII as well as COLII and COLX in human adult articular chondrocytes, and also increased the levels and nuclear localization of SMADs1/5/8, while TGFβ1 showed minimal or no activation of the chondrogenic program in adult chondrocytes. Targeted blockade of CaMKII with specific siRNAs decreased levels of pSMADs, COLII, COLX and proteoglycans in normal and OA adult articular chondrocytes in the presence of both BMP4 and TGFβ1. Both human fetal and PSC-derived chondrocytes also demonstrated a decrease of chondrogenic differentiation in the presence of small molecule and peptide inhibitors of CaMKII. Furthermore, immunoprecipitation for SMADs1/5/8 or 2/3 followed by western blotting for pCaMKII showed direct interaction between SMADs and pCaMKII in primary chondrocytes. CONCLUSION: Current study demonstrates a direct role for CaMKII in TGF-β and BMP-mediated responses in primary and PSC-derived chondrocytes. These findings have direct implications for tissue engineering of cartilage tissue from stem cells and therapeutic management of OA.

Scientific Abstract:
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